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TITLE: Treatment of Prostate Cancer by Targeting Vascular Endothelial Growth Factor Receptors (VEGFRs) and Micrometastases with Bismuth-213 Labeled Vectors

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14. ABSTRACT
The main purpose of the proposed study was to evaluate the toxicity and efficacy of multiple targeting vectors for the treatment of prostate cancer in mouse models. After successfully achieving the in vitro outcomes, the in vivo studies have also proven to be a great success. The efficacy of the proposed combination therapy has proven to be far better than the mono-therapy and the results are significantly different. Various combination therapy regimes were well tolerated in mice whereas the long-term toxicity studies are currently ongoing. The dose optimization, time interval optimization and subcutaneous efficacy studies have been completed whereas orthotopic model studies are expected to be complete in three months. Thus the in vitro (radiolabeling of Avastin, in vitro stability, enhancement of plasminogen activation expression and estimation of VEGF secretion by various prostate cancer cell lines) and in vivo studies have gone as per expectations and plan.

15. SUBJECT TERMS
Tumour model development, anti-VEGF monoclonal antibody Bevacizumab, combination therapy, radiolabeling, radioimmunoconjugate, stability, uPA expression, PAI2, Bismuth-213, tumour area, In vivo efficacy, In vivo toxicity

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INTRODUCTION

The Award involves the combination of the anti-vascular endothelial growth factor (VEGF) therapy with targeted alpha therapy (TAT). It combines the existing TAT skills of the PI with another targeting approach (anti-VEGF therapy) that has been used against various cancers. A brief introduction that was provided in the annual report earlier is copied below:

Solid tumours require their own vascular systems for progressive growth and the production of angiogenic growth factors by tumour cells is necessary to induce their functional tumour vasculature[1,2]. VEGF is one of the most important growth factors and possesses strong angiogenic activity in both in vitro and in vivo assays and also is responsible for the formation of vascular hyperpermeability, ascites and edema[3,4]. The presence of this angiogenic growth factor in a number of tumours has been demonstrated, and VEGF expression is up-regulated in hypoxic tumour areas[5-7] and is expressed at high levels in other cancers[8-11]. In prostate cancer, the degree of tumour vascularization correlates with the development of metastatic disease[12]. Though the critical factors that promote angiogenesis in prostate cancer are yet to be defined, Melnyk et.al[13] found that VEGF promotes tumour angiogenesis in prostate cancer. Utilizing a VEGF-neutralizing monoclonal antibody[14], they examined the effect of VEGF inhibition in an in vivo model of metastatic prostate cancer. They found that inhibition of VEGF suppresses both primary prostatic tumour growth and dissemination of micrometastases. In addition, they also found that VEGF appeared to be inhibiting further growth and metastatic progression of well established tumour grafts. For prostate cancer, however, the anti-VEGF therapy has not been too successful and it needed to be combined with other therapeutic approaches and TAT was considered as one of them. The PI proposed to use the FDA approved and humanized anti-VEGF monoclonal antibody, Avastin, for this purpose. The proposal comprises a comprehensive regime of in vitro and in vivo experiments to test the proposed hypothesis that stated "Vascular endothelial growth factor receptors (VEGFRs) are expressed by most prostate cancer patients that can be targeted by the commercially available anti-VEGF monoclonal antibody labelled with Bismuth-213 alpha emitter and the remainder can be eliminated by Bismuth-213 labeled plasminogen activator inhibitor type 2 (PAI2)". Targeting has been the main pillar of the study, but the approach is two-fold. Blocking the VEGFRs by anti-VEGF therapy (labelled and unlabeled) inhibits tumour proliferation and this phenomenon will be tested in combination with TAT.

BODY

The two-year study required a series of Tasks to be performed with an appropriate timeline for each Task. Below is a description of the work carried out to-date and is based on the approved Statement of Work (SOW) tasks and milestones as outlined in the proposal. The yearly report submitted earlier provides details on the accomplishment of various experimental objectives with particular reference to certain tasks as proposed in the original research proposal. A brief summary of these completed tasks is provided below:

Task 1 Test and develop the methods for the in vitro experiments that includes testing of the prostate cancer cell lines (PC3, LN3-LNCaP and DU145) for VEGF secretion, detection of an enhancement of plasminogen activation (PA) expression by VEGF and labelling of Avastin with Bismuth-213.

Progress / Accomplishments The Task was completed within the time frame proposed.

Experimental Objectives achieved The following experimental objectives as proposed in the original protocol were achieved by completing this Task 1:

1. In vitro measurements of the VEGF concentration in the prostate cancer cells that will be used for the in vitro and in vivo experiments of the proposed study and to develop an immunohistological procedure for the detection of VEGFRs in these cell lines by using Avastin.

2. To determine whether VEGF enhances the plasminogen activation (PA) expression in 3 cell lines.

3. To standardize the labeling procedure for Avastin with ^{213}Bi.

[***Experimental Objectives 1-3 achieved***].

Task 2 ***Development of the animal models*** The animal models will be chosen based on the in vitro experiments for the detection of VEGF and VEGFR. All three cell lines have been reported to secrete VEGF and VEGFR and if similar results are found, I plan to use all three models for the efficacy and toxicity of the combination therapy. Time line for development of animal models is 4-6 months.

Progress / Accomplishments The Task was completed within the time frame proposed as the animal models were successfully developed and the PI received adequate training by Professor Pam Russell (one of the Mentors) prior to the actual cell inoculations. This included anaesthesia and recovery protocols, operating and locating the prostate, administration of appropriate volumes of saline in prostate and possible

complications and common errors that are made during such procedures. Training for correct stitching of the two layers was also provided.

Experimental Objectives achieved The following experimental objectives as proposed in the original protocol were achieved by completing this Task 2

1. To develop appropriate tumour xenograft models required for the study based on VEGF secretion and VEGFR expression by the cell lines tested in vitro.

[***Experimental Objective 4 achieved***].

Task 3 The efficacy and toxicity of Avastin has been reported in the literature. However, we will perform this experiment to compare it with Bismuth-213 labeled Avastin. This task does not involve the combination therapy and only one tumour model will be used. Timeline for the completion of the task and achievement of the milestone is 6-12 months.

Progress / Accomplishments The Task was started within the time frame proposed in the protocol, but the final results are still being worked out as longer monitoring was required for some groups.

The yearly report contained information to this stage as this particular experiment was still going on at the time of the submission of that report. The experiment and a series of other experiments proposed have now been completed. This final report thus starts from this Task and goes on further to the remaining Tasks as proposed.

Task 3 Continued The task required a comparison of the efficacy for unlabeled and ^{213}Bi-labeled Avastin. For the practical reasons, the task was split in two parts with first part being the optimization of the Avastin dose and the second part being the comparison of the ^{213}Bi-Avastin with that of unlabeled Avastin in terms o efficacy.

In the first part three different doses of cold Avastin were administered in mice. The doses included a range from low (1 mg/kg) to high (5 mg/kg) with a medium range of 3 mg/kg. The tumour size of the mice at the start of Avastin administration ranged between 2-4 mm in any one direction [the range is provided for information only – the minimum tumour volume in each mouse was ensured to be at least 2 mm at the start of the Avastin therapy]. All mice were initially administered with the specified doses of cold Avastin at twice weekly for three weeks from the day of the first administration.

One group of mice received appropriate dose of Avastin twice weekly over three weeks (6 doses) after which the Avastin administration was stopped. It was however, continued for another group that received two additional doses over another week i.e 8 doses in total spread over 4-weeks at twice weekly administrations. Tumour area (mm^2) was measured by cross-sectional measurements of the tumour size in each direction during the entire length of the experiment. The following Figures 1 and 2 show the effect of respective therapy with respect to tumour growth overtime.

Figure 1 Tumour Area (mm^2) for Cold Avastin Dose Optimization Studies with 6 doses over 3 weeks

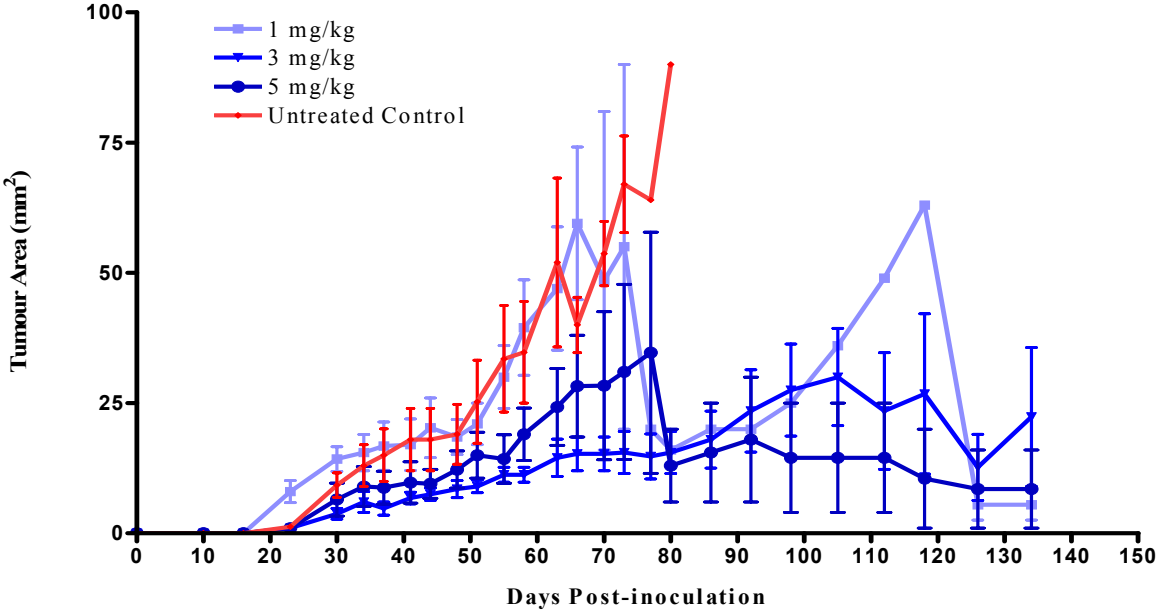
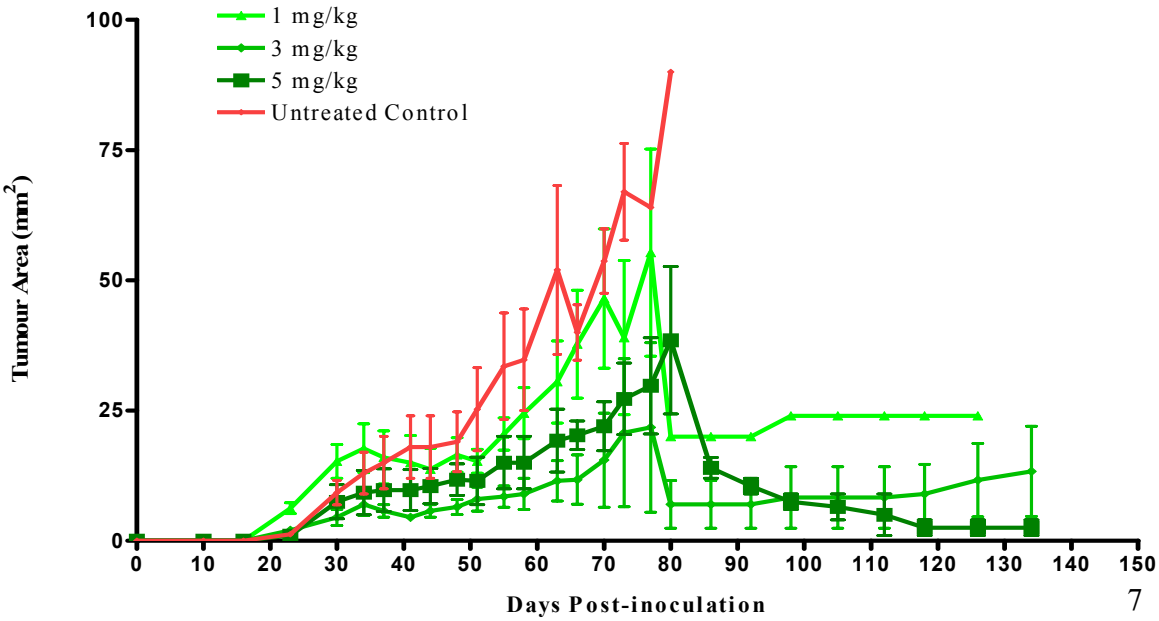


Figure 2 Tumour Area (mm^2) for Cold Avastin Dose Optimization Studies with 8 doses over 4 weeks



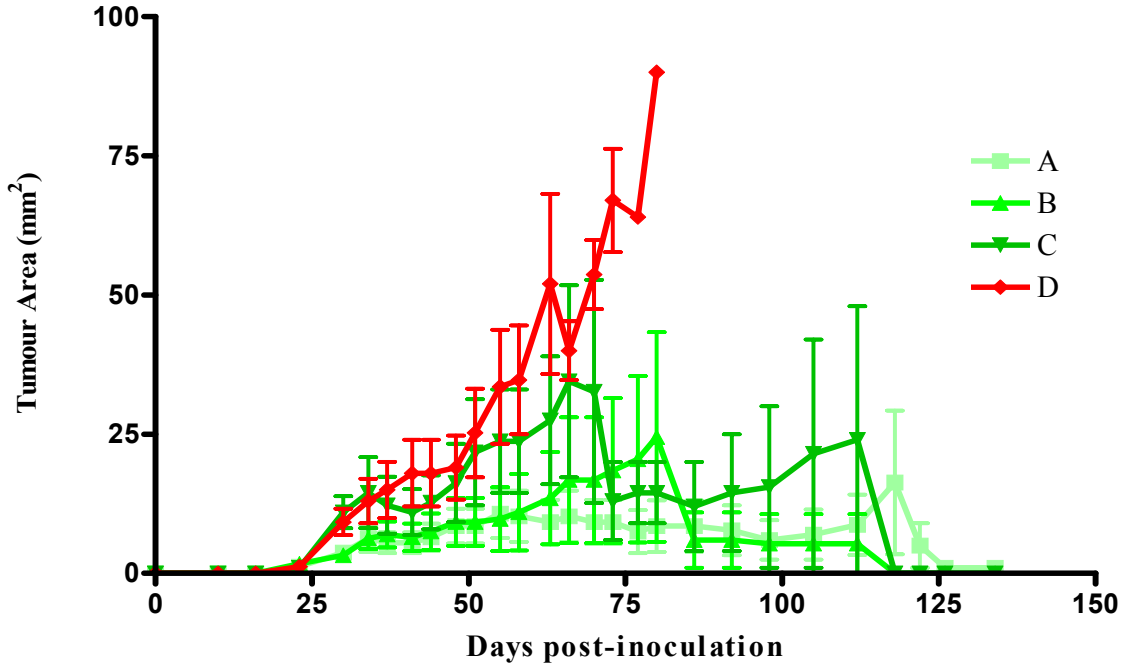
Figures 1 and 2 clearly demonstrate the dose dependent efficacy of Avastin as 5 mg/kg shows a significantly slower tumour growth compared to 1 mg / kg dose (*p*-value <0.05). Mean tumour area for the three tested doses of Avastin for 6 dose treatments was 24.8±3.6, 12.8±1.7 and 13.7±1.8 mm^2 for 1, 3 and 5 mg / kg doses respectively compared to 29.1±6.0 mm^2 for the controls. The Figure 2 shows a more pronounced effect of Avastin when the treatment was continued for two additional doses over one week i.e. 8 Avastin doses in total. This effect was however non-significant (*p*-value 0.459) compared to 6 dose treatments. The mean tumour area for 8 dose treatments was 21.2±2.6, 8.3±1.0 and 11.4±1.9 mm^2 for 1, 3 and 5 mg / kg doses respectively compared to 29.1±6.0 mm^2 for the controls.

In the 2nd part of this proposed Task, experiments were carried out to compare the efficacy of labeled and unlabeled Avastin. For this purpose, the following three combinations of labeled and unlabeled Avastin were carried out:

1. 1 mg / kg Avastin followed by one single injection of 9 mCi/kg ^{213}Bi-Avastin.
2. 3 mg / kg Avastin followed by one single injection of 9 mCi/kg ^{213}Bi-Avastin.
3. 5 mg / kg Avastin followed by one single injection of 3 mCi/kg ^{213}Bi-Avastin.

Note *The Avastin administrations irrespective of the dose were carried out over three weeks at twice weekly administrations i.e. 6 doses in total. The single administration of ^{213}Bi-Avastin was carried out at 4-days after the administration of the last Avastin dose.*

Figure 3 Comparison of the three different combinations of Avastin and ^{213}Bi-Avastin combination therapy



Results of this experiment are given in Figure 3 where in the legend:

A represents 1 mg / kg Avastin followed by 9 mCi / kg ^{213}Bi-Avastin, B is 3 mg / kg Avastin followed by 9 mCi / kg ^{213}Bi-Avastin, C is 5 mg / kg Avastin followed by 3 mCi / kg ^{213}Bi-Avastin combination therapy.

Figure 3 clearly demonstrates the efficacy of the combination therapy compared to the controls with the *p*-value being <0.05. The mean tumour area for the three combination therapy groups was 6.5±0.7, 7.5±4.8 and 13.3±9.3 mm^2 for A, B and C respectively compared to 29.1±6.0 mm^2 for the controls.

These results suggest that the mice that received the lowest (1 mg / kg) Avastin in combination with highest ^{213}Bi-Avastin (9 mCi / kg) showed the best of efficacy results with a mean tumour volume of 6.5±0.7. It also showed that the difference between the 3 mg / kg and 5 mg / kg doses was not significant although it was significant compared to controls. Combination therapy was found to be more effective than Avastin alone therapy.

Accomplishments The Task was started within the time frame proposed in the protocol but the follow-up and periodic measurements (weight and tumour volume) delayed the final results. The efficacy of Avastin alone and that of the Avastin / ^{213}Bi-Avastin combination therapy was successfully established. The dose optimizations and the first of the combination therapy experiments were completed.

Experimental Objectives Achieved The following experimental objectives as proposed in the original protocol were achieved by completing this Task 3.

1. To determine the efficacy and toxicity of cold Avastin and ^{213}Bi-Avastin in one tumour model.

[Experimental Objective 5 achieved].

Task 4 In the initially submitted proposal, this Task was stated as under,

"The efficacy and toxicity of the combination therapy, comprising cold Avastin and Bismuth-213 labeled PAI2, will be tested in one tumour model as this experiment does not include Bismuth-213 labeled Avastin. Timeline for the completion of this task is 12-18 months".

The task required a comparison of the efficacy for unlabeled and the second of the targeting vectors proposed in the study i.e. ^{213}Bi-labeled PAI2. To complete this proposed Task, experiments were carried out to compare the efficacy of the combination therapy of the proposed vectors. For this purpose, the following three combinations of the vectors were carried out:

1. 1 mg / kg Avastin followed by one single injection of 9 mCi/kg ^{213}Bi-PAI2.

2. 3 mg / kg Avastin followed by one single injection of 9 mCi/kg ^{213}Bi-PAI2.
3. 5 mg / kg Avastin followed by one single injection of 9 mCi/kg ^{213}Bi-PAI2.

Note *The Avastin administrations irrespective of the dose were carried out over three weeks at twice weekly administrations i.e. 6 doses in total. The single administration of ^{213}Bi-PAI2 was carried out at 4-days after the administration of the last Avastin dose.*

Results of this experiment are given in Figure 4 where in the legend:

A represents 1 mg / kg Avastin followed by 9 mCi / kg ^{213}Bi-PAI2, B is 3 mg / kg Avastin followed by 9 mCi / kg ^{213}Bi-PAI2, C is 5 mg / kg Avastin followed by 9 mCi / kg ^{213}Bi-PAI2 combination therapy.

Figure 4 Comparison of the three different combinations of Avastin and ^{213}Bi-PAI2 combination therapy

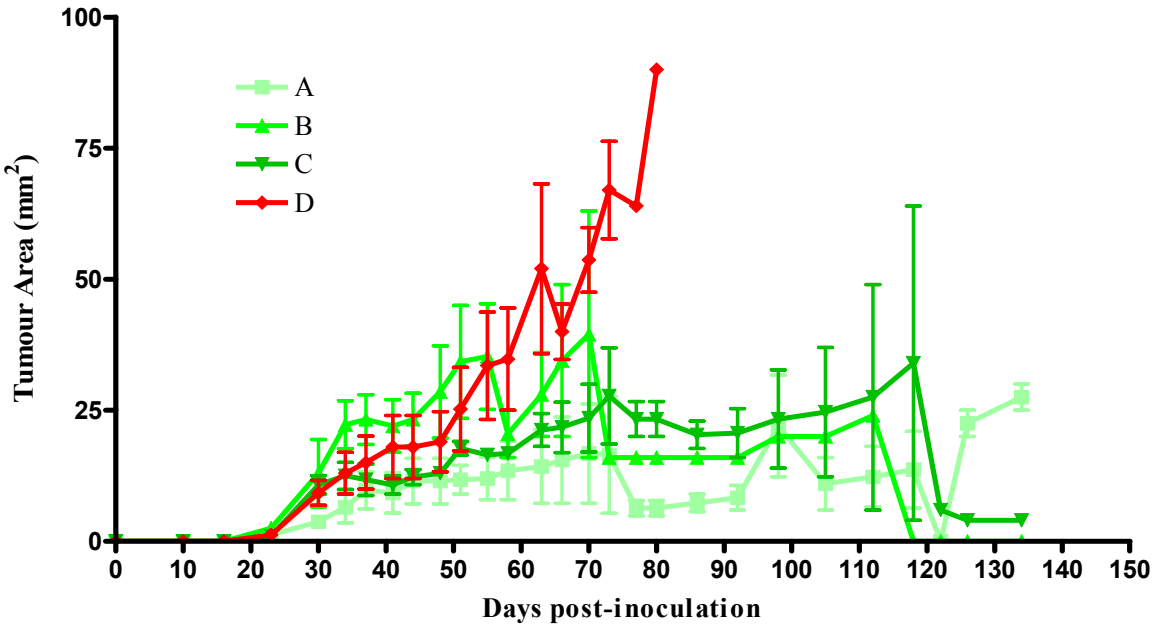


Figure 4 clearly demonstrates the efficacy of the combination therapy compared to the controls with the *p*-value being <0.05. The mean tumour area for the three combination therapy groups was 10.4±1.3, 16.8±2.4 and 15.3±1.8 mm^2 for A, B and C respectively compared to 29.1±6.0 mm^2 for the controls.

These results suggest that the mice that received the lowest (1 mg / kg) Avastin in combination with highest ^{213}Bi-PAI2 (9 mCi / kg) showed the best of efficacy results with a mean tumour volume of 10.4±1.3. It also showed that the difference between the 3 mg / kg and 5 mg / kg doses was not significant although it was significant compared to

controls. Combination therapy was found to be more effective than Avastin alone therapy.

Accomplishments The accomplishment of the Task was delayed due to a number of reasons beyond control. The efficacy of Avastin alone and that of the Avastin / ^{213}Bi-PAI2 combination therapy was successfully established.

Experimental Objectives Achieved The following experimental objective as proposed in the original protocol were achieved by completing this Task 4.

1. To determine the efficacy and toxicity of the combination therapy of the optimized dose of cold Avastin (from Experiment 5) and varying doses of ^{213}Bi-PAI2 in one tumour model.

[Experimental Objective 6 achieved].

Task 5 In the initially submitted proposal, this Task was stated as under,

"This includes optimization of the time interval between the Bismuth-213 labeled Avastin and Bismuth-213 labeled PAI2 using one tumour model as for Tasks 3 and 4.two therapies using Bismuth-213. Timeline for the completion of the task and achievement of the milestone is 9-15 months".

The Task required the establishment of an optimized time interval between the Avastin only and the Avastin / ^{213}Bi-Avastin combination therapy so as the entire therapeutic regime proposed remains as safe as possible. Since the overall doses for either of the radiolabeled vectors i.e. Avastin and PAI2 were kept constant (even if split dose administrations were carried out), a separate experiment for ^{213}Bi-PAI2 was neither proposed nor carried out. The following Table 1 provides information about the experimental design for this experiment.

Table 1 Time-line for time course optimization study

Dose		**Days post-last of Avastin dose administration**
Avastin (mg/kg)	**^{213}Bi-Avastin (mCi/kg)**	
1	**3**	**4**
		7
		10
		14
5	**9**	**4**
		7
		10
		14

Thus four different time points were tested for the administration of ^{213}Bi-Avastin after the unlabeled Avastin therapy was stopped. Please note that the experimental parameters were kept constant i.e. all mice received 6 doses of Avastin at twice weekly and ^{213}Bi-Avastin was administered after 4, 7, 10 and 14 days of the last Avastin administration. Due to experimental reasons, only the low (1 mg / kg Avastin and 3 mCi / kg ^{213}Bi-Avastin) and high (5 mg / kg Avastin and 9 mCi / kg ^{213}Bi-Avastin) dose studies were carried out [this resulted in the exclusion of 5 mg / kg Avastin dose only]. Figures 5 and 6 present the data for low and high dose studies.

Figure 5 Tumour Area (mm^2) for Avastin / ^{213}Bi-Avastin Combination Therapy for Time Course Optimization

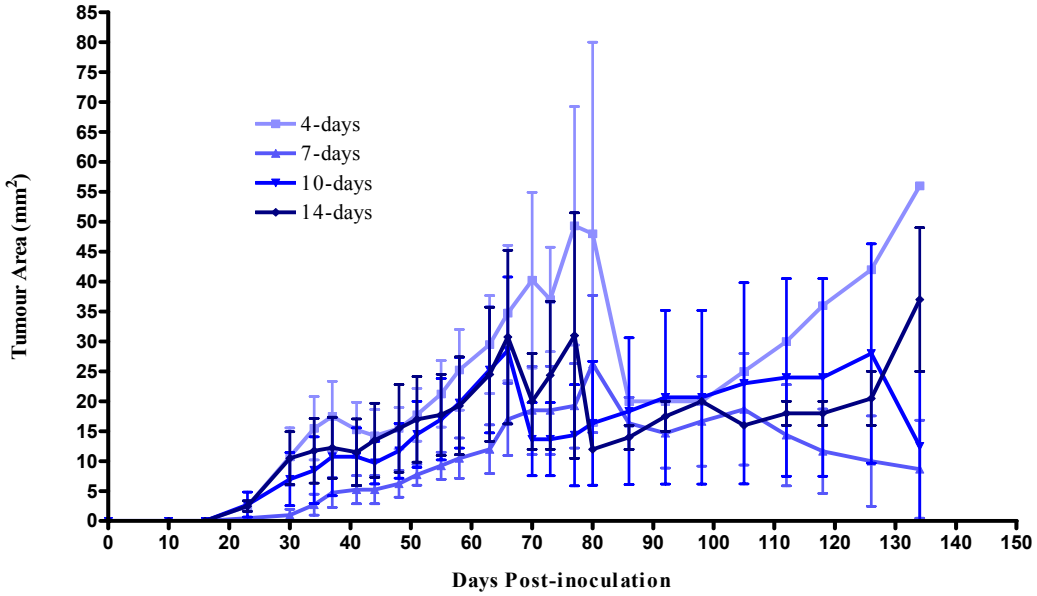
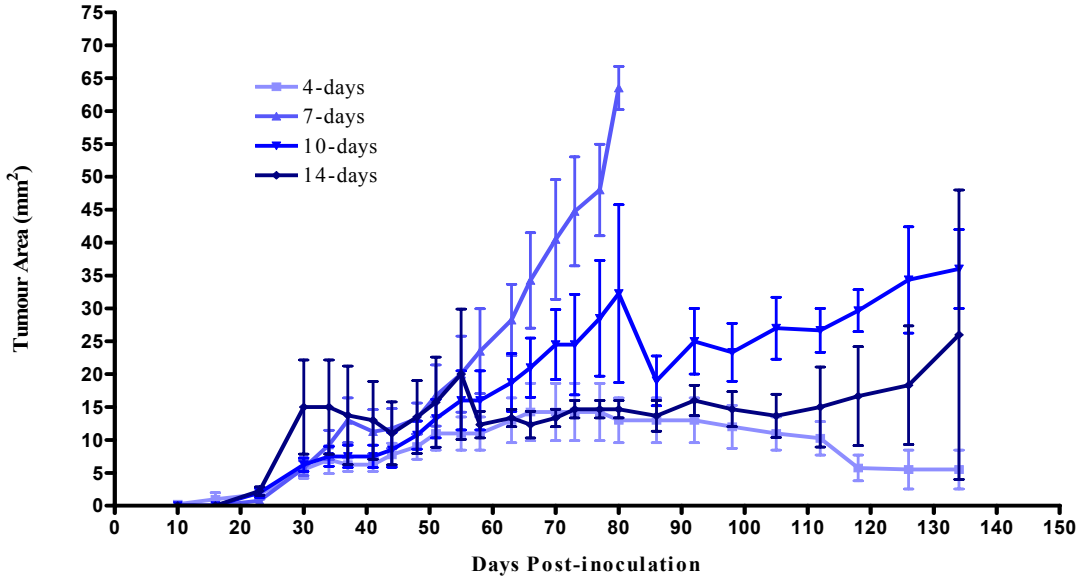


Figure 6 Tumour Area (mm^2) for Avastin / ^{213}Bi-Avastin Combination Therapy for Time Course Optimization



Analyses of the data from this time course experiment shows that the time interval between the administration of unlabeled and labeled Avastin might have a substantial impact on the final outcome of the study. It is clearly evident from Figure 5 that 4-day time point is not the appropriate time for such sequential combination therapy studies as the mean tumour area was found to be 23.8±3.0 mm^2 compared to 10.21±1.4, 14.7±1.6 and 16.1±1.8 mm^2 for the 7, 10 and 14 days time points respectively. With high dose time course analyses however, it is a totally different scenario. The 4-day time point provides the best result with a tumour area of 9.1±0.8 mm^2 compared to 21.4±4.3, 17.9±2.1 and 13.4±1.1 mm^2 for the 7, 10 and 14 days time points respectively. This data also suggests that for high dose efficacy studies with combination therapy, 4-days time point remains the best time to administer the radiolabeled vector. This is also in line with our previous studies with ^{213}Bi-labeled vectors. From this experiment and based on our previous experience, it was decided that 4-days time point will be used for future experiments in the proposed study.

Accomplishments The accomplishment of the Task was achieved in time as the required experiments were carried out ahead of the ones required for Task 4 and together with the ones required for Task 3. The time interval optimization was successfully achieved by accomplishing this proposed Task.

Experimental Objectives Achieved The following experimental objective as proposed in the original protocol were achieved by completing this Task 5.

1. To determine the optimized time interval between ^{213}Bi-Avastin and ^{213}Bi-PAI2 administration for combination therapy using one tumour model.

[Experimental Objective 7 achieved].

Task 6 In the initially submitted proposal, this Task was stated as under,

"This includes the efficacy and toxicity of Bismuth-213 labeled Avastin therapy followed by Bismuth-213 PAI2 therapy at the optimized time interval between the two therapies. Since this experiment tests the ultimate objective of the study, it will be carried out on all tumour models chosen on the basis of Task 1. Timeline for the completion of this task is 15-21 months".

This Task required the use of optimized parameters on all i.e. subcutaneous and orthotopic tumour models. The subcutaneous model work has already been completed and is reported here. The orthotopic model work has not yet been carried out due to certain reasons beyond control. This includes the late start of the entire in vivo work as a new animal holding facility was being built in our building and while it has now been operational, now it is the scheduling of the radionuclide that is delaying the start of this experiment (that requires 6-8 weeks for completion).

The experiment included the combination therapy using all the three vectors. For this purpose experiments were carried out using the following combinations of the vectors:

1. 1 mg / kg Avastin followed by one single injection of 1.5 mCi / kg ^{213}Bi-Avastin and 1.5 mCi / kg ^{213}Bi-PAI2.
2. 1 mg / kg Avastin followed by one single injection of 4.5 mCi / kg ^{213}Bi-Avastin and 4.5 mCi / kg ^{213}Bi-PAI2.
3. 3 mg / kg Avastin followed by one single injection of 1.5 mCi / kg ^{213}Bi-Avastin and 1.5 mCi / kg ^{213}Bi-PAI2.
4. 3 mg / kg Avastin followed by one single injection of 4.5 mCi / kg ^{213}Bi-Avastin and 4.5 mCi / kg ^{213}Bi-PAI2.
5. 5 mg / kg Avastin followed by one single injection of 1.5 mCi / kg ^{213}Bi-Avastin and 1.5 mCi / kg ^{213}Bi-PAI2.
6. 5 mg / kg Avastin followed by one single injection of 4.5 mCi / kg ^{213}Bi-Avastin and 4.5 mCi / kg ^{213}Bi-PAI2.

Note *The Avastin administrations irrespective of the dose were carried out over three weeks at twice weekly administrations i.e. 6 doses in total. The administration of ^{213}Bi-labeled vectors was carried out at 4-days after the administration of the last Avastin dose.*

Figure 7 provides a graphic presentation of the data for combination therapy. The ***"Legend"*** for the Figure 7 is as follows:

A represents 1 mg / kg Avastin followed by one single injection of 1.5 mCi / kg ^{213}Bi-Avastin and 1.5 mCi / kg ^{213}Bi-PAI2.

B represents 1 mg / kg Avastin followed by one single injection of 4.5 mCi / kg ^{213}Bi-Avastin and 4.5 mCi / kg ^{213}Bi-PAI2.

C represents 3 mg / kg Avastin followed by one single injection of 1.5 mCi / kg ^{213}Bi-Avastin and 1.5 mCi / kg ^{213}Bi-PAI2.

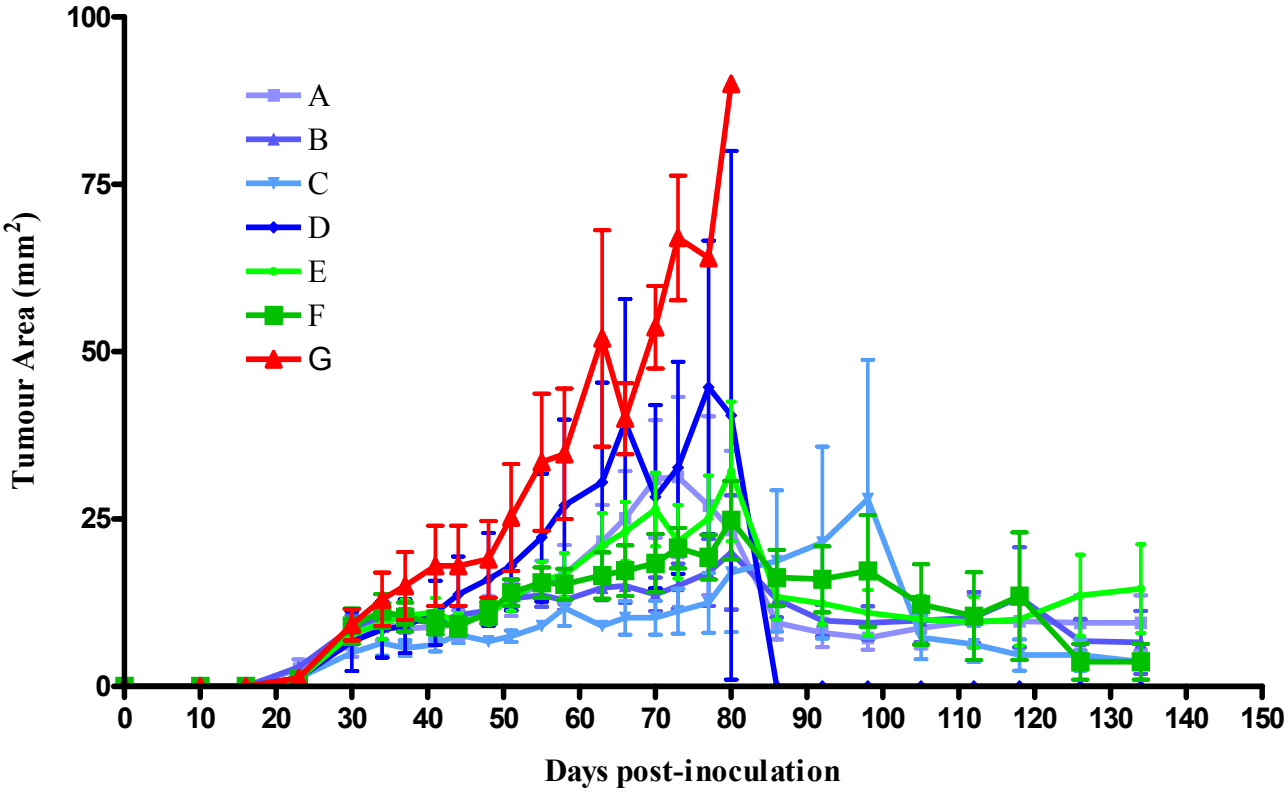
D represents 3 mg / kg Avastin followed by one single injection of 4.5 mCi / kg ^{213}Bi-Avastin and 4.5 mCi / kg ^{213}Bi-PAI2.

E represents 5 mg / kg Avastin followed by one single injection of 1.5 mCi / kg ^{213}Bi-Avastin and 1.5 mCi / kg ^{213}Bi-PAI2.

F represents 5 mg / kg Avastin followed by one single injection of 4.5 mCi / kg ^{213}Bi-Avastin and 4.5 mCi / kg ^{213}Bi-PAI2.
G represents untreated control mice inoculated at the same time as the treated mice.

These data clearly show a significant advantage of all forms of combination therapy compared to control group. It is evident that all dose groups have shown significant (*p*-value <0.01) regression in terms of tumour growth and development compared to controls. The mean tumour area for the groups A – G is 12.3±1.7, 10.3±1.0, 8.6±1.3, 12.9±2.9, 12.9±1.6, 11.7±1.3 and 29.1±5.9 mm^2 respectively. It can therefore, be concluded that combination therapy offers substantial advantages over mono-therapy with Avastin alone.

Figure 7 Combination therapy results plotted against days post-inoculation



Accomplishments The accomplishment of the Task was achieved with some delay due to reasons mentioned earlier. The primary objective of the proposal was to test the hypothesis that Avastin in combination with targeted alpha therapy (TAT) will be of great advantage compared to its use as a single agent. This set of experiments proves it to be correct.

Experimental Objectives Achieved The following experimental objective as proposed in the original protocol were achieved by completing this Task 6.

1. To determine the efficacy and toxicity of the optimized combination therapy of ^{213}Bi-Avastin (Experiment 6) and ^{213}Bi-PAI2 in all tumour models.

[Experimental Objective 8 achieved].

NOTE ***One experiment with orthotopic tumour model still remains to be carried but we are expecting it to begin in late April 2008 and finish by June 2008 [an addendum to this report will be submitted on completion of that experiment].***

Toxicity Studies As per proposal, the toxicity studies were carried out in two ways. The above experiments were also considered for toxicity with periodic weight measurements and activity / behaviour recordings. The Figures showing weights of these mice are available and can be submitted, if required. In brief, the administered doses ae well tolerated in mice and while there is an early loss of weight, it is reversible and mice return to normal weight within 2-week period. The second of the toxicity experiments proposed was the long-term toxicity. This experiment is currently ongoing and will be continued until the orthotopic model combination therapy efficacy experiment is completed [or as long as practically possible]. From this ongoing long-term toxicity experiment, the biochemistry / haematology data and histopathology data for various times points is already available [since the data is incomplete, it is not presented at this stage].

KEY RESEARCH ACCOMPLISHMENTS

The key research accomplishments to-date are given below:

- Successful standardization of a method for the quantitative estimation of Vascular Endothelial Growth Factor (VEGF) secreted by three prostate cancer cell lines.

- Enhancement of uroplasminogen activation (uPA) expression has successfully demonstrated.

- Tumour models (subcutaneous and orthotopic) have been successfully established.

- The major success / accomplishment has been the successful labeling of Avastin with the alpha emitting radionuclide Bi-213 and testing the stability of the so-produced radioimmunoconjugate in vitro [a paper has been submitted to "Cancer Biology and Therapy" and result is being awaited].

- The time interval between cold and radiolabeled Avastin has been successfully established.

- The combination therapy dose and time-interval optimization experiments have successfully been achieved.

- The combination therapy has been proven to be better than the mono-therapy using one vector only. Ongoing and remaining studies will further confirm this phenomenon.

REPORTABLE OUTCOMES

The major reportable outcome has been the successful labeling and testing of the anti-VEGF monoclonal antibody Avastin. The specific activity work is being carried out alongside the in vivo stability studies. The labeling data however, in itself has resulted in a full-fledged refereed research paper that has been submitted to an International refereed journal and a result is being awaited at this stage.

The results of the completed experiments and ongoing research work clearly demonstrate the superiority of the multiple targeting achieved in case of cold and hot Avastin, cold Avastin and ^{213}Bi-PAI2 and the hot Avastin and ^{213}Bi-PAI2 combination therapies compared to Avastin only (cold or hot) and ^{213}Bi-PAI2 only mono-therapies.

CONCLUSION

Avastin has been and thus can be successfully labeled with radionuclides that can be used to enhance the efficacy and targeting of cold Avastin. Combination therapy certainly seems to be a better option compared to Avastin based mono-therapy [as evidenced from the ongoing animal studies].

It is however, recommended that following studies should be carried out to ensure validity and completeness of the data with respect to the initiation of early Phase clinical trials for prostate cancer using the proposed therapy:

Optimization of specific activity (increased specific activity will require less amounts of the antibody and thus the target blocking effect of the antibody will be reduced resulting in an increased targeting by the antibody),

The accuracy of pharmacokinetics studies is extremely important and we have time and again observed that such studies with the short half-lived ^{213}Bi provide only a general trend rather than accurate pharmacokinetics. It is therefore, suggested that ^{205}Bi / ^{206}Bi radionuclide be used for these studies (preliminary experiments have already been carried out and results are being analysed),

Toxicity studies should essentially be carried out in a second species and it is proposed by the medical oncologists in our Institution that rabbits be used for this purpose. A data from mice and rabbits for acute, short-term and long-term toxicity will provide sound basis for a human clinical trial.

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